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Antiretroviral Therapy With Protease Inhibitors in Human Immunodeficiency Virus Type 1– and Human Herpesvirus 8–Coinfected Patients

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Human herpesvirus 8 (HHV-8) is believed to play a role in the pathogenesis of Kaposi's sarcoma (KS) and possibly in other proliferative disorders often associated with human immunodeficiency virus type 1 (HIV-1) infection. Recent case reports have indicated resolution of KS and clearance of HHV-8 DNA from peripheral blood mononuclear cells (PBMC) in HIV-1–infected subjects following highly effective antiretroviral therapy, including HIV-1 protease inhibitors (PI), suggesting a possible activity for these compounds on HHV-8 replication. In the present study, the time course of PBMC HHV-8 DNA levels, plasma HIV-1 RNA load, and $CD4^+$ T-cell counts were followed up in six coinfected subjects (four with and two without KS) under antiretroviral therapy with PI. A specific anti–HHV-8 role for PI was not consistently found, since fluctuation of HHV-8 viral load over time appeared to be independent of treatment. Nevertheless, our data support the hypothesis that KS patients may significantly benefit from PI therapy as an indirect consequence of partial restoration of immune functions following effective anti–HIV-1 combination therapy. ***J. Med. Virol. 57:140–144, 1999.***

KEY WORDS: HIV-1; antiretroviral therapy; HHV-8; Kaposi's sarcoma

INTRODUCTION

The human herpesvirus type 8 (HHV-8) has been suggested to play a role in the pathogenesis of Kaposi's sarcoma (KS) and possibly in other proliferative disorders such as multicentric Castleman's disease (MCD) and body cavity-based lymphomas [reviewed by Cesarman and Knowles, 1997]. While HHV-8 is regularly detected in affected tissues, cross-sectional studies have also shown that peripheral blood mononuclear cells (PBMC) from the majority of patients with HHV-8–related disorders and occasionally from healthy subjects may harbor HHV-8 DNA [Ambroziak et al., 1995; Bigoni et al., 1996; De Milito et al., 1996]. While the epidemiology and pathology of HHV-8 have not yet been fully elucidated, HHV-8 infection has been mainly documented in HIV-1–infected subjects. Factors accounting for the association between HHV-8 and HIV-1 infection include a common route of homosexual transmission [De Milito et al., 1996; Martin et al., 1998; Melbye et al., 1998] and possibly reactivation or upregulation of HHV-8 replication in the presence of HIV-1–induced underlying immunosuppression [Verbeek et al., 1998].

A growing number of HIV-1–infected patients are being treated with the currently licensed HIV-1 protease inhibitors (PI) saquinavir (SQV), ritonavir (RTV), indinavir (IDV), and nelfinavir (NFV), often resulting in a dramatic decrease in HIV-1 viremia and a general improvement of clinical status [Carr and Cooper, 1996]. Case reports have been recently published indicating resolution or regression of AIDS-associated KS [Blum et al., 1997; Conant et al., 1997; Murphy et al., 1997; Rizzieri et al., 1997] and clearance of HHV-8 DNA from PBMC [Blum et al., 1997; Rizzieri et al., 1997] following antiretroviral therapy with PI. However, the development of mucosal KS has been reported in a patient following RTV and SQV therapy [Weir and Wansbrough-Jones, 1997] and no clinical or virological effect of IDV therapy was shown in a patient with MCD [Dupin et al., 1997]. Indeed, the suggestion that HIV-1 PI may play some direct anti–HHV-8 role has not been formally proved.

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In order to assess the potential role of HIV-1 PI both on KS and on HHV-8 DNA load in PBMC, the clinical and virological course of HIV-1 and HHV-8 infection were examined in six coinfected subjects, four of whom were affected with KS, following combined antiretroviral therapy, including reverse trascriptase inhibitors and PI.

MATERIALS AND METHODS

The PBMC population was purified from citrated whole blood by centrifugation over a Ficoll-Hypaque (Pharmacia Biotech, Uppsala, Sweden) density gradient and DNA was extracted by a salting-out method. Briefly, 5–10 × 10^6 PBMC were resuspended in 0.4 ml of lysis buffer (10-mM Tris-HCl, pH 8.2, 400-mM NaCl, 2-mM EDTA, 0.2% SDS, 400 μg/ml proteinase K) and incubated for 1 hr at 56°C, followed by addition of 400 μl of 4-M NaCl, vortexing and centrifugation at 1,600 × g for 15 min. The supernatant containing the DNA was transferred to another tube and DNA was precipitated with isopropanol, collected by centrifugation, washed with 70% ethanol, resuspended in TE buffer (10-mM Tris-HCl, pH 8, 0.2-mM EDTA), and evaluated spectrophotometrically. All DNA samples were confirmed to be suitable for PCR analysis by successful amplification of HIV-1 DNA targets [Zazzi et al., 1993].

One microgram of DNA was used as the template for nested PCR detection of HHV-8 DNA as previously described [Whitby et al., 1995]. Given the high sensitivity of the optimized nested PCR procedure, samples that did not yield the expected HHV-8 PCR product were considered to contain <1 copy of HHV-8 DNA per microgram. A semiquantitative PCR amplification was then performed on HHV-8–positive samples by using an external standard curve. The PCR-amplified HHV-8 $KS330_{233}$ region [Chang et al., 1994] was cloned into plasmid pCRII by using the TA-Cloning system (Invitrogen, Leek, the Netherlands). The resulting plasmid $pKS330_{233}$ was linearized at a unique *Sal*I site, purified by ethanol precipitation, and quantitated by spectrophotometry and agarose gel electrophoresis. All of the HHV-8–positive samples to be titrated were amplified in the same 40-cycle single-round PCR run used to generate the standard titration curve with 10^1, 10^2, 10^3, 10^4, and 10^5 copies of $pKS330_{233}$ template DNA (Fig. 1).

PCR products were evaluated by densitometric scanning of ethidium bromide-stained bands on agarose gels using Sigmagel (Jandel Scientific, Erkrath, Germany). The experiment was repeated twice, yielding a number of copies differing less than fourfold for each clinical sample. DNA samples that did not produce a detectable HHV-8 band in this assay were considered to contain <10 copies HHV-8 DNA per microgram. Plasma HIV-1 RNA load was quantitated by using HIV-1 Amplicor assay (Roche Molecular Systems, Branchburg, NJ).

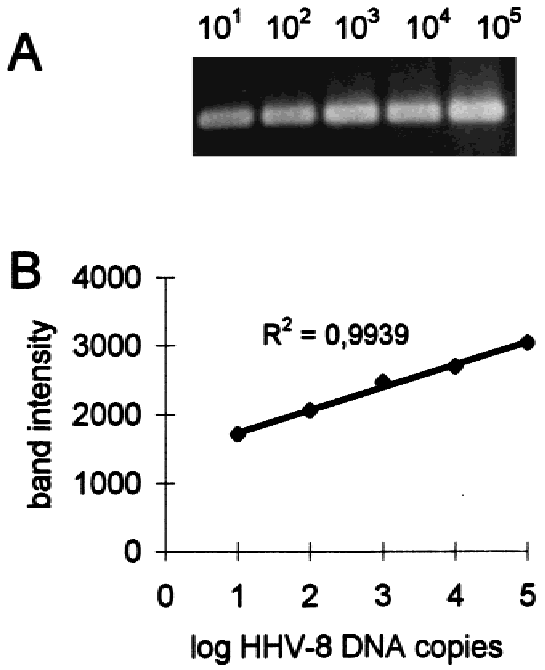

Fig. 1. Construction of the standard curve for HHV-8 DNA quantification. PCR products obtained from 10^1 to 10^5 copies of cloned HHV-8 target sequence (**A**) were measured densitometrically and plotted against the number of template copies (**B**). Clinical samples were amplified in the same PCR run used to generate the standard titration curve and titrated by interpolation. The experiment was repeated twice yielding a mean $R^2 = 0.9920$ and copy numbers differing less than fourfold for each clinical sample.

RESULTS

Figure 2 shows antiretroviral therapy and time course of HIV-1 plasma RNA, HHV-8 PBMC DNA, and $CD4^+$ T-lymphocyte counts for the six HIV-1– and HHV-8–coinfected patients studied.

Patient 1 was a woman with cutaneous KS. Following surgical treatment of the first lesion, new multiple cutaneous KS lesions appeared 6 months later and progressed through the following 8 months. Vinblastine and interferon treatment was then started concomitant with ganciclovir and foscarnet therapy for cytomegalovirus retinitis. Following these treatments, partial and stable regression of KS lesions was obtained. Seven months later the patient was started on RTV therapy that resulted in further improvement of KS lesions and a >2.0 log reduction in HIV-1 viremia. Undetectable levels of plasma HIV-1 RNA were maintained throughout the follow-up period. A high HHV-8 DNA load (2,755 genomes/μg DNA) was transiently detected in PBMC only at month 7 with respect to initiation of RTV therapy. All of the other samples obtained since month -1 through month 11 did not contain detectable levels of HHV-8 DNA.

Patient 2, a man without KS, received IDV therapy after relatively ineffective antiretroviral therapies with different nucleoside reverse transcriptase inhibitors. The patient was shown to harbor comparable amounts of HHV-8 DNA at three time points but HHV-8 was

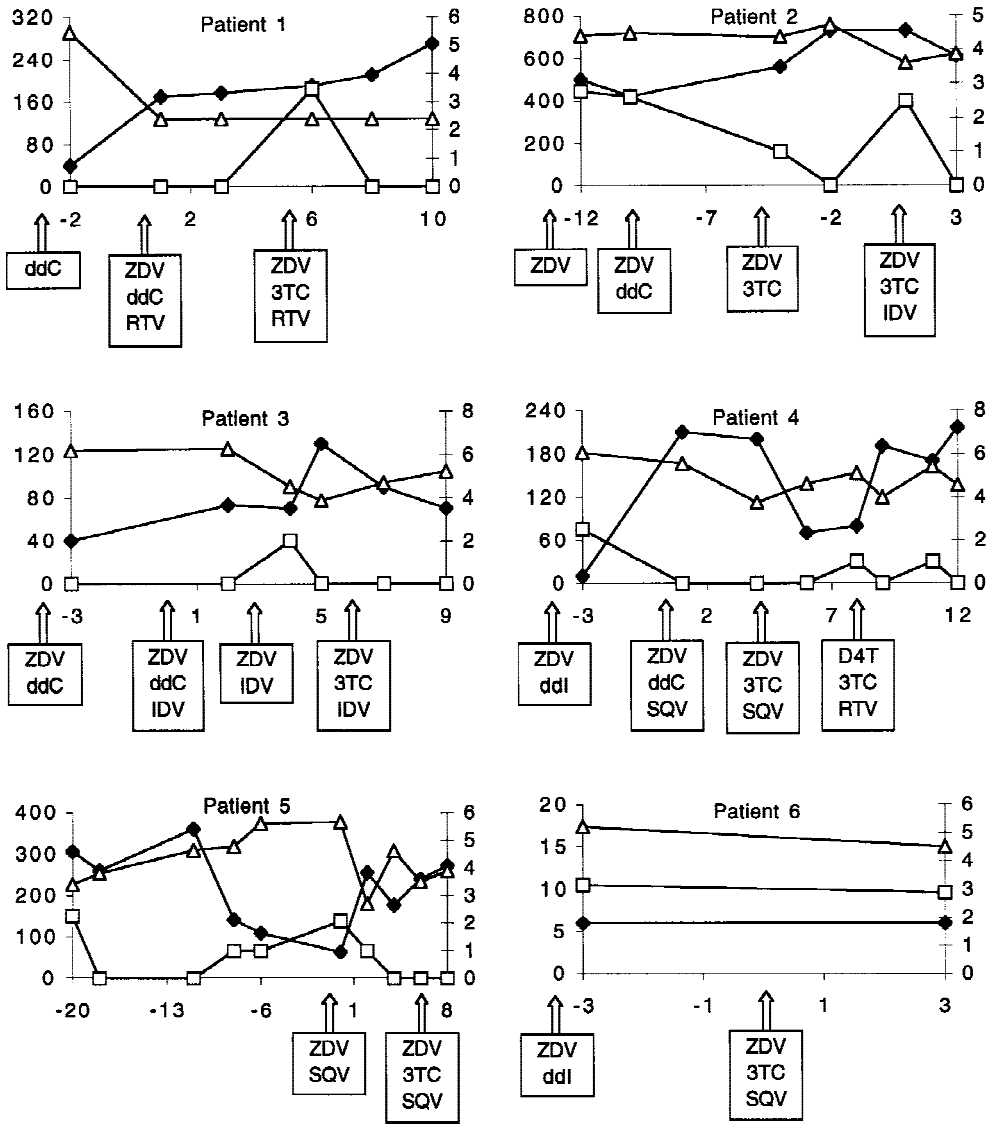

Fig. 2. Antiretroviral therapy and time course of plasma HIV-1 RNA levels (-△-), HHV-8 DNA load in PBMC (-□-), and CD4+ cell counts (-♦-) in the six HIV-1– and HHV-8–coinfected subjects analyzed. The X-axis shows the time course (months) with respect to initiation of PI therapy; CD4+ cell counts (number/mm^3) are indicated on the left Y-axis; and HIV-1 RNA and HHV-8 DNA levels are reported in a logarithmic scale on the right Y-axis. Antiretrovirals are abbreviated as follows: ZDV, zidovudine; ddI, didanosine; ddC, zalcitabine; d4T, stavudine; 3TC, lamivudine; SQV, saquinavir; RTV, ritonavir; IDV, indinavir.

intermittently undetectable during the follow-up period. While IDV appeared to reduce HIV-1 viral load, a temporal association between treatment with the HIV-1 protease inhibitor and decrease in HHV-8 DNA burden could not be demonstrated.

Patient 3 was a homosexual male with AIDS who developed a single forearm cutaneous KS lesion 1 month after starting IDV therapy. Following surgical removal of this lesion no relapse or appearance of new lesions occurred. HIV-1 viral load was very high at the time of diagnosis of KS and decreased substantially, although transiently, only after 2 months of treatment with IDV and following discontinuation of ddC due to neurotoxicity. HHV-8 DNA was detected in PBMC at a single time point when the patient had been treated with IDV for 4 months.

Patient 4 was a homosexual male with severely disseminated KS. Significant and stable regression of both visceral metastasis and cutaneous lesions was obtained following antineoplastic chemotherapy and shift to anti–HIV-1 therapy with zidovudine, zalcitabine/lamivudine, and SQV. Due to early rebounding HIV-1 RNA titers, anti–HIV-1 therapy was subsequently changed, substituting stavudine and RTV for lamivudine and SQV. HHV-8 DNA was measurable 1 month before starting SQV therapy but decreased to undetectable or very low levels thereafter, consistent with the absence of progression of KS lesions.

Patient 5, a homosexual male without KS, was shown to be intermittently positive for HHV-8 DNA for several months before commencing SQV therapy. Treatment with SQV was temporally associated with a

progressive decrease of HHV-8 load to undetectable levels, concomitant with a significant reduction in HIV-1 viremia.

Patient 6 was a homosexual male with very low $CD4^+$ cell counts and severely disseminated cutaneous KS. Three months after SQV therapy there was a modest decline in HIV-1 RNA load, without any clinical improvement of KS lesions. Likewise, sustained levels of HHV-8 DNA were found in PBMC from this patient both before and after SQV treatment.

DISCUSSION

Several case reports and one cohort study have been described in which possible effects of combined antiviral therapy with PI were evaluated both on HHV-8 and AIDS-related KS [Blum et al., 1997; Conant et al., 1997; Murphy et al., 1997; Rizzieri et al., 1997; Weir and Wansbrough-Jones, 1997; Lebbé et al., 1998; Parra et al., 1998]. We report a clinical and virological follow-up study of six HIV-1– and HHV-8–coinfected patients following addition of PI to the antiretroviral therapy. Regression of KS lesions after initiation of combined therapy with PI was observed in two of the four AIDS-KS patients analyzed (patients 1 and 4) and lack of reappearance of KS in patient 3 may also have been related to IDV therapy. Indeed, although in this patient KS developed after addition of PI to the therapy, the delay in decreasing HIV-1 RNA load suggests initially poor adherence to IDV therapy.

By contrast, no effect of SQV treatment was shown on the severe cutaneous KS in patient 6 in the limited follow-up period. Our findings are in agreement with previous reports indicating regression of KS and HHV-8–related disorders in most [Blum et al., 1997; Conant et al., 1997; Murphy et al., 1997; Rizzieri et al., 1997; Parra et al., 1998] but not all [Dupin et al., 1997; Weir and Wansbrough-Jones, 1997] patients after treatment with PI. However, it must be noted that resolution of KS in our patients may have been favored by other anti-KS treatments, including antineoplastic chemotherapy (patients 1 and 4), surgery (patients 1 and 3), and antiherpetic drugs (patient 1).

On the other hand, a clear association was not observed between PI therapy and HHV-8 DNA load in PBMC. Indeed, HHV-8 DNA levels appeared to fluctuate over time both during (patients 1, 2, 3, and 4) and before (patients 2 and 5) PI therapy. Previous reports have also documented inherent fluctuations of HHV-8 DNA in PBMC [De Milito et al., 1996] or its appearance after initiation of PI therapy [Weir and Wansbrough-Jones, 1997]. However, the possibility that PI therapy specifically reduces HHV-8 replication in KS lesions, as suggested by a single case report [Blum et al., 1997], remains to be fully evaluated. Indeed, a decrease of HHV-8 DNA load in KS lesions has been reported in two out of three patients under antiretroviral therapy with PI, although for one of the patients the HHV-8 DNA load in KS lesions appeared to fluctuate during the PI therapy [Lebbé et al., 1998].

Since KS likely results from a complex interaction of viral and immunological factors, the favorable effects of PI therapy on AIDS-related KS may be also explained by factors other than HHV-8 load, including decrease in HIV-1 replication and restoration of immune functions [Blum et al., 1997; Murphy et al., 1997; Lebbé et al., 1998; Parra et al., 1998]. Accordingly, we observed both decreasing HIV-1 RNA levels and rising $CD4^+$ cell counts in all three patients in whom regression of KS was observed following PI therapy (patients 1, 3, 4) but not in patient 6, in whom KS did not respond to SQV treatment.

It is interesting to note that a temporal correlation between increasing $CD4^+$ cell numbers and falling HHV-8 DNA levels was also apparent in patients 2, 3, 4, and 5, consistent with the hypothesis that $CD4^+$ lymphocytes play a role in controlling HHV-8 replication [Whitby et al., 1995; Bigoni et al., 1996; Lebbé et al., 1998]. Our finding is in agreement with a recent report in which clinical response to the combination therapy with PI in AIDS-KS patients correlated with increased $CD4^+$ cell number [Lebbé et al., 1998]. Indeed, combined antiretroviral therapy was shown to improve reactivity of $CD4^+$ T-cells to recall antigens [Autran et al., 1997]. Thus, the suggestion that HIV-1 PI directly control HHV-8 replication and HHV-8–related disorders may be interpreted in terms of partial restoration of the immune system following effective anti–HIV-1 therapy, including PI rather than specific anti–HHV-8 activity of these compounds. Since the effects of HIV-1 PI on HHV-8 and HHV-8–related disorders have been so far reported only in anecdotal studies, we consider advisable to review a more substantial number of cases to clarify the actual impact of PI therapy on HHV-8 pathology. Antiretroviral treatments of comparable potency were not available prior to development of PI, making it unlikely to obtain indirect remission of KS from anti–HIV-1 therapy. Indeed, presently available and ongoing anti–HIV-1 protease inhibitors may be of significant clinical benefit for AIDS patients suffering from KS as well as from other diseases induced by viruses that may be favored by underlying immunosuppression and/or upregulated by HIV-1 itself.

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